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Effect of grape seed extract on growth performance, protein and polyphenol digestibilities, and antioxidant activity in chickens

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Abstract

Grape seed extract (GSE) provides a concentrated source of polyphenols that have antioxidant capacity. An experiment was conducted to investigate the effect of GSE at levels 0.6, 1.8 and 3.6 g kg^{-1} in broiler chicks (1 to 42 days) on growth performance, relative weights (pancreas, spleen and liver) and lengths (duodenum, jejunum, ileum and ceca) of digestive organs, relative weights of liver fat and abdominal fat, ileal protein digestibility, excreta extractable polyphenol digestibility, and antioxidant activity of diet and excreta. The inclusion of GSE did not affect the performance and the relative liver and pancreas weights. Relative intestinal length was reduced at 21 days of age by the inclusion of GSE. The inclusion of GSE caused an increase of relative spleen weight at 42 days of age. Ileal digestibility of crude protein was increased at 21 days of age in birds fed GSE diets. Excreta extractable polyphenol digestibility was increased at 21 and 42 days of age by the inclusion of GSE in the diets reaching values of 57 to 65% and 60 to 69%, respectively. Antioxidant activity in GSE diets and excreta exhibited higher scavenging free radical capacity at 21 and 42 days than control diet. We concluded that GSE could be a new source of antioxidant in animal nutrition.

Additional key words: flavonoids, relative organ sizes.

Resumen

Efecto del extracto de semilla de uva sobre los índices productivos, la digestibilidad de la proteína y los polifenoles y la actividad antioxidante en pollos broiler

El extracto de semilla de uva (ESU) posee una gran capacidad antioxidante debido a su riqueza en polifenoles. Se ha llevado a cabo un experimento para investigar el efecto de la inclusión de ESU a concentraciones de 0,6, 1,8 y 3,6 g kg^{-1} en raciones de pollos broiler, administradas hasta los 42 días de edad, sobre los índices productivos, el peso relativo del páncreas, bazo e hígado, las longitudes relativas del duodeno, yeyuno, ileon y ciegos, los pesos relativos de la grasa del hígado y de la grasa abdominal, la digestibilidad ileal de la proteína, la digestibilidad de los polifenoles extractables en la excreta y la actividad antioxidante en la dieta y en la excreta. La inclusión de ESU no modificó los índices productivos y el peso relativo del hígado y el páncreas. La longitud relativa intestinal estaba disminuida a los 21 días de edad por la inclusión de ESU. La inclusión de ESU causaba un incremento del peso relativo del bazo a los 42 días de edad. La digestibilidad ileal de la proteína bruta estaba incrementada a los 21 días de edad en aves alimentadas con ESU. La digestibilidad fecal de los polifenoles extractables estaba incrementada a los 21 y 42 días de edad con la incorporación de ESU en las dietas alcanzando valores de 57 a 65% y 60 a 69%, respectivamente. La actividad antioxidante de las dietas y la excreta de las aves alimentadas con ESU mostraron una mayor capacidad quelante sobre los radicales libres a los 21 and 42 días que la dieta control. Basados en estas observaciones podemos concluir que el ESU puede ser un nueva fuente de antioxidante que podría ser utilizado en nutrición animal.

Palabras clave adicionales: flavonoides, tamaño relativo de los órganos.

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Abbreviations used: ABTS [2, 2-azinobis (3-ethilenzotiazolin)-6-sulfonate], AIA (acid-insoluble ash), AID (apparent ileal digestibility), CP (crude protein), EP (extractable polyphenols), GSE (grape seed extract), NEP (non extractable polyphenols).

Introduction

Research for new bio-efficient antioxidants has particularly focused on natural antioxidants to respect the consumer concerns over safety and toxicity. Grape seeds and by-products of wine/grape juice processing provide an abundant source of flavonoids, in which the most abundant classes include the flavan-3-ols. Grape seeds from grape juice and wine processing can be separated, extracted, dried and purified into grape seed extract (GSE) which contain polyphenolic compounds. Nutritional interest in polyphenolic compounds has increased greatly in light of their antioxidant activity (Scalbert and Williamson, 2000), but there have been very few studies on the digestibility and intestinal degradation of polyphenols and other major constituents. It is noteworthy that most reports on the beneficial effects of polyphenols have been obtained from *in vitro* studies, and more detailed investigations are required to extrapolate these results to *in vivo* situations. In this sense, clinical data has shown the antioxidant potential of grape seed (Shi *et al*., 2003).

The antioxidant compounds present in grape have been identified as phenolic acids (benzoic and hydroxycinnamic acids), stilbene derivatives, flavan-3-ols (catechin and epicatechin), flavonols (quercetin and myricetin), and anthocyanidins (Caillet *et al*., 2006). Shi *et al*. (2003) reported that the antioxidant potential of grape seed is twenty and fifty fold greater than vitamins E and C, respectively arising from increased levels of polyphenols proanthocyanidins and oligomers of flavan-3-ol units, especially catechin and epicatechin present in GSE (Yilmaz and Toledo, 2004). The antioxidant activity of GSE has been reported to improve the oxidative stability in a variety of food systems including cooked beef (Ahn *et al.*, 2002), turkey and pork patties, and cold stored turkey meat (Lau and King, 2003; Mielnik *et al*., 2006; Carpenter *et al*., 2007). However, the use of such natural antioxidants in animal nutrition could be limited to the low bioavailability of polyphenols.

Previous experiments in our laboratory (Goñi *et al*., 2007; Brenes *et al*., 2008) have shown an increase in the antioxidant activity of broiler diet, excreta, and meat as a result of the dietary administration of grape pomace concentrate. The objective of the present study was to assess the effect of increasing dietary concentrations of a commercial grape seed extract on the performance parameters, protein, and extractable polyphenols digestibilities, and the antioxidant activity in diet and excreta of chickens.

Materials and methods

A total of 240, 1-day-old male broiler Cobb chicks, were housed in electrically heated starter batteries in an environmentally controlled room. The chicks were allocated to 40 pens, each pen containing six chicks, to receive four dietary treatments with ten replicates of each treatment during 21 d. Diets in mash form and water were provided ad libitum. At 3 weeks of age, 20 pens were randomly selected with six birds per pen and five pens per treatment, and moved to grower-finisher batteries during the rest of 21 d of experimental period (21-42 d). Celite (Celite Corp., Lompoc, CA 93436) a source of acid insoluble ash (AIA) was added at 10 g kg^{-1} to all diets as an indigestible marker. Experimental procedures were approved by the University Complutense of Madrid Animal Care and Ethics Committee in compliance with the Ministry of Agriculture, Fishery and Food for the Care and Use of Animals for Scientific Purposes. Ingredients and nutrient composition of diets are shown in Table 1. Experimental diets were as follows: (1) control corn-soybean diet (CS); (2) CS+ 0.6 g kg^{-1} grape seed extract (GSE); (3) CS + 1.8 g kg^{-1} GSE; (4) CS+ 3.6 g kg^{-1} GSE. The GSE contained 95% of total polyphenols of which 38-42% was catechin and total proanthocianidins (composition as stated by the manufacturer- Naturex, Avignon, France).

Collection of samples and measurements

At 21 and 42 d of age the chicks (20 and 10 randomly selected chicks respectively per treatment, 2 per replicate) were sacrificed by cervical dislocation and liver, pancreas, spleen and abdominal fat were weighed and the length of duodenum, jejunum, ileum and ceca were measured. The ileum was quickly dissected out and the content expressed by gentle manipulation into a plastic container and stored at –20°C. Digesta were pooled from two birds of each replicate within the same treatment. Diet and ileal contents were freeze-dried and ground (1 mm screen) and subsequently analysed for N-Kjeldahl and celite. Clean stainless steel collection trays were also placed under each cage and excreta from the birds were collected for 48 h. A subsample of excreta was collected in polyethylene bags and freeze-dried for subsequent determination of extractable polyphenols (EP), and antioxidant activity.

Table 1. Ingredients and nutrient composition of experimental diets (g kg^{-1} as fed)

Ingredients	Grower (1-21 days)	Finisher (22-42 days)
Corn (8.1% CP)	521.4	641.2
Soybean (48% CP)	379.1	286.1
Sunflower oil	45.7	26.2
Dicalcium phosphate	18.0	18.7
Calcium carbonate	15.8	8.9
Salt	3.0	3.0
Vitamin-mineral premix[1]	5.0	5.0
DL- Methionine	1.8	1.0
Celite[2]	10.0	10.0
Grape seed extract (GSE)[3]	±	±
Analyzed composition		
Crude protein	230	195
Extractable polyphenols[4]	0.09-0.18-0.29-0.40	0.16-0.26-0.38-0.49
Calculated composition		
AME[5] (kcal kg^{-1})	3,100	3,025
Methionine + cystine	8.8	8.8
Ca	10.0	10.0
Available P	4.5	3.7

[1] Vitamin and mineral mix supplied the following per kilogram of diet: vitamin A, 8,250 IU; cholecalciferol, 1,000 IU; vitamin E, 11 IU; vitamin K, 1.1 mg; vitamin B_{12}, 12.5 µg; riboflavin, 5.5 mg; Ca panthotenate, 11 mg; niacin, 53.3 mg; choline chloride, 1020 mg; folic acid, 0.75 mg; biotin, 0,25 mg; delquin, 125 mg; DL-methionine, 500 mg; amprol, 1 g; Mn, 55 mg; Zn, 50 mg; Fe, 80 mg; Cu, 5 mg; Se, 0.1 mg; I, 0.18 mg; NaCl, 2,500 mg. [2] Celite Corp, Lompoc, CA 93436. [3] GSE: 0, 0.6, 1.8 and 3.6 g kg^{-1}. [4] Corresponding to inclusion levels of 0, 0.6, 1.8 and 3.6 g kg^{-1} GSE, respectively. [5] AME: apparent metabolisable energy; calculated values (FEDNA Tables, 2003).

Chemical analysis

Crude protein in diet and ileal content was analysed according to the methods of the AOAC (1995). The AIA contents of diet, ileal digesta and excreta were measured after ashing the samples and treating the ash with boiling 4 M HCl (Siriwan *et al*., 1993). Diet and excreta were extracted by constant shaking at room temperature with a methanol solution (50:50 v/v, 50 mL g^{-1} sample during 60 min). After centrifugation (15 min, 3,000 g) supernatants were combined and used to measure the antioxidant capacity by the ABTS [2, 2-azinobis (3-ethilenzotiazolin)-6-sulfonate] method. ABTS is one of the most frequently used methods based on the generation of the highly stable chromophoric cation-radical of $ABTS^{+}$. This enables determinations to be performed easily and quickly, allowing a considerable number of samples to be processed together and it provides very reliable results (Miller *et al*., 1996). This antioxidant activity was estimated following the procedure described by Re *et al*. (1999) with some modifications. ABTS radical cation ($ABTS^{+\cdot}$) was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark at room temperature for 12-16 h before use. The $ABTS^{+\cdot}$ solution was diluted with methanol to an absorbance of 0.70 ± 0.02 at 658 nm. After addition of 100 µL of extracted samples or Trolox standard to 3.9 mL of diluted $ABTS^{+\cdot}$ solution, absorbance reading were taken every 20 s using a Beckman DU-640 ([3]Beckman Instruments Inc, Fullerton, CA). The reaction was monitored for 6 min. The percentage inhibition of absorbance *vs.* time was plotted and the area bellow the curve (0-6 min) was calculated. Methanolic solutions of known Trolox concentrations were used for calibration the measurement of EP antioxidant activity. Extractable polyphenols were determined in methanol/acetone/water extracts obtained from diet and excreta by Folin-Ciocalteau procedure (Montreau, 1972) using gallic acid as standard

Calculations and statistical analysis

Apparent ileal crude protein (CP) and excreta extractable polyphenols (EP) digestibilities were calculated using the following formula:

$$100\% - \left[100\% \times \left(\frac{AIA\ in\ feed}{AIA\ in\ digesta\ or\ excreta} \times \frac{CP\ and\ EP\ in\ digesta\ or\ excreta}{CP\ and\ EP\ in\ feed}\right)\right]$$

Data were subjected to analysis of variance using the General Linear Models (GLM) procedures of SAS (2001), with treatment comparisons among the control and the GP diets. Linear and quadratic effects were also analyzed. Significant differences among treatment means were determined at $P < 0.05$ by Duncan's multiple-range test.

Results

Growth performance

The addition of increasing concentration of GSE in the chicken diets did not change the growth performance, feed consumption and feed to gain ratio (Table 2) at 21 and 42 days of age compared with those birds fed control diet.

Relative organ sizes

The inclusion of graded concentrations of GSE did not affect the relative organ weights (pancreas, liver, liver fat and abdominal fat) except in the case of spleen which was increased up to 14% at 42 days of age in birds fed with the highest concentrations of GSE compared with those fed control diet (Tables 3 and 4). Regarding relative organ lengths, the inclusion of graded concentration of GSE caused a significant decrease of jejunum (up to 18%; linear and quadratic effect), ileum (up to 19%; linear effect) and ceca (up to 9%; linear effect) lengths at 21 days of age. However, at 42 days of age, an increase in the relative lengths of jejunum (6%), ileum (9%) and ceca (14%) was only observed in birds fed the highest GSE concentration in the diet (Tables 3 and 4).

Protein and extractable polyphenol digestibilities

The inclusion of graded concentration of GSE caused a significant increase of ileal protein digestibility (up to 4%; linear effect) at 21 days of age and extractable polyphenol digestibility in excreta at 21 (up to 11%; linear and quadratic effects) and 42 days (up to 13%; linear and quadratic effects) of age compared with those birds fed control diet (Table 5).

Antioxidant activity in diets and excreta

The addition of increasing concentration of GSE caused a significant increase of antioxidant activity in grower (up to 388%, linear and quadratic effects) and

Table 2. Performance of broiler chicks fed diets containing grape seed extracts (GSE)

Treatments	0-21 days[1]			0-42 days[2]		
	Weight gain (g)	Feed consumption (g)	Feed to gain ratio	Weight gain (g)	Feed consumption (g)	Feed to gain ratio
Control	635	819	1.29	2,279	3,993	1.75
C + 0.6 GSE	666	835	1.25	2,215	3,854	1.74
C + 1.8 GSE	651	833	1.28	2,289	3,979	1.74
C + 3.6 GSE	623	791	1.27	2,172	3,773	1.74
Pooled SEM	45.7	56.8	0.04	99.3	171.4	0.03
Statistical significance (P-value of contrast)						
Control *vs.* GSE	NS[3]	NS	NS	NS	NS	NS
Type of response due to percentage GSE in diet						
Linear	NS	NS	NS	NS	NS	NS
Quadratic	NS	NS	NS	NS	NS	NS

[1] Data are means of 10 pens of 6 chicks. [2] Data are means of 5 pens of 6 chicks. [3] NS = $P > 0.05$.

Table 3. Relative weights and lengths of digestive organs of broiler chicks fed grape seed extracts (GSE)[1] at 21 days of age

Treatments	Relative weight[1] (%)			Relative length[1] (%)			
	Pancreas	Spleen	Liver	Duodenum	Jejunum	Ileum	Ceca
Control	0.29	0.095	2.77[a]	3.38	7.68[a]	7.68[a]	1.82[a]
C + 0.6 GSE	0.28	0.088	2.71[ab]	3.30	6.55[b]	6.40[b]	1.73[ab]
C + 1.8 GSE	0.29	0.095	2.81[a]	3.15	6.55[b]	6.25[b]	1.66[b]
C + 3.6 GSE	0.29	0.106	2.48[b]	3.37	6.27[b]	6.56[b]	1.70[ab]
Pooled SEM	0.04	0.02	0.26	0.37	0.55	0.62	0.12
Statistical significance (P-value of contrast)							
Control *vs.* GSE	NS[2]	NS	NS	NS	0.001	0.001	0.01
Type of response due to percentage GSE in diet							
Linear	NS	NS	NS	NS	0.001	0.001	0.05
Quadratic	NS	NS	0.05	NS	0.01	NS	NS

[a-b] Means in columns with no common superscript differ significantly ($P < 0.05$). [1] Data are means of 20 chicks for each treatment. [2] NS = $P > 0.05$.

finisher diets (up to 417%, linear and quadratic effects). Likewise, the inclusion of graded concentration of GSE caused a significant increase of antioxidant activity in excreta at 21 (up to 61%; linear and quadratic effects) and 42 (up to 77%; linear and quadratic effects) days of age compared with those birds fed control diet (Table 5).

Discussion

The present study demonstrated that the inclusion of concentrations of GSE up to 3.6 g kg^{-1} did not change the growth performance (0 to 3 weeks and 3 to 6 weeks of age). Few data are available in the literature in relation to the use of grape polyphenol extracts in chicken feed. Hughes *et al.* (2005) and Lau and King (2003) reported a growth depression with the use of grape seed extract containing 90.2% of total phenolics, expressed as gallic acid equivalent by the Folin method, and incorporated in the diet at 30 g kg^{-1}. In the current experiment, GSE contained 45.5% extractable polyphenols but it was included in a concentration of 3.6 g kg^{-1} in the chicken diets. Evidently the total polyphenols present in the higher concentration in our study is relatively low to produce a growth depression effect.

Table 4. Relative weights and lengths of digestive organs of broiler chicks fed grape seed extracts (GSE)[1] at 42 days of age

Treatments	Relative weight[1] (%)					Relative length[1] (%)			
	Pancreas	Spleen	Liver	Liver fat	Abdominal fat	Duodenum	Jejunum	Ileum	Ceca
Control	0.16[ab]	0.106[bc]	1.98	5.49	0.91	1.30[a]	2.89[ab]	2.97[b]	0.74[c]
C + 0.6 GSE	0.16[ab]	0.100[c]	1.95	5.77	0.86	1.28[a]	2.72[b]	2.98[b]	0.81[ab]
C + 1.8 GSE	0.18[a]	0.121[a]	2.06	5.42	0.91	1.17[b]	2.69[b]	2.88[b]	0.77[bc]
C + 3.6 GSE	0.15[b]	0.119[ab]	2.08	5.42	0.94	1.26[a]	3.05[a]	3.24[a]	0.84[a]
Pooled SEM	0.01	0.02	0.19	0.59	0.16	0.09	0.24	0.29	0.07
Statistical significance (P-value of contrast)									
Control *vs.* GSE	NS[2]	NS	NS	NS	NS	NS	NS	NS	NS
Type of response due to percentage GSE in diet									
Linear	NS	0.013	NS	NS	NS	NS	NS	NS	0.011
Quadratic	NS	NS	NS	NS	NS	NS	0.001	0.040	NS

[a-c] Means in columns with no common superscript differ significantly ($P < 0.05$). [1] Data are means of 10 chicks for each treatment. [2] NS = $P > 0.05$.

Table 5. Ileal digestibility of protein, excreta extractable polyphenols digestibility and antioxidant activity of extractable polyphenols in diet and excreta of broilers chicks fed grape seed extracts (GSE) at 21 and 42 days of age

Treatments	Ileal protein digestibility (%)		Excreta extractable polyphenols digestibility (%)		Antioxidant activity (μmol Trolox equivalent g^{-1})			
					Diet		Excreta	
	21 days[1]	42 days[2]	21 days[3]	42 days[4]	1-21 days	22-42 days	21 days[3]	42 days[4]
Control	84.2[b]	84.3	58.9[b]	61.0[b]	3.36[d]	4.2[d]	76.7[d]	77.9[d]
C + 0.6 GSE	86.1[ab]	85.8	57.2[b]	60.8[b]	6.27[c]	7.8[c]	96.5[c]	101.1[c]
C + 1.8 GSE	85.7[ab]	85.6	57.9[b]	61.3[b]	9.84[b]	10.4[b]	117.6[b]	121.6[b]
C + 3.6 GSE	87.6[a]	84.2	65.4[a]	68.9[a]	16.41[a]	21.7[a]	123.8[a]	138.2[a]
Pooled SEM	1.55	1.49	1.79	1.16	0.09	0.17	1.90	1.98
Statistical significance (P-value of contrast)								
Control *vs.* GSE	0.01	NS[5]	NS	NS	0.001	0.005	0.001	0.001
Type of response due to percentage GSE in diet								
Linear	0.001	NS	0.001	0.001	0.001	0.001	0.001	0.001
Quadratic	NS	NS	0.001	0.001	0.01	0.001	0.001	0.001

[a-d] Means in columns with no common superscript differ significantly ($P < 0.05$). [1] Data are means of 10 samples corresponding to 20 birds. [2] Data are means of 5 samples corresponding to 10 birds. [3] Data are means of 10 pens of 6 chicks each. [4] Data are means of 5 pens of 6 chicks each. [5] NS = $P > 0.05$.

These results agree with previous reports conducted in our lab (Goñi *et al*., 2007; Brenes *et al*., 2008) using grape pomace concentrate. However, Jansman *et al*. (1989) and Ortiz *et al*. (1993) observed a growth depression in chickens fed sorghum and faba bean based diet, respectively due to the higher polyphenol concentrations, mainly condensed tannins, of these ingredients.

The presence of polyphenolic compounds in diet may have some adverse metabolic effects which are mainly associated with lower efficiency of nutrients particularly protein, inhibition of digestive enzymes and increased excretion of endogenous protein. Polyphenols bind to protein due to the interaction of their reactive hydroxyl groups with the carbonyl groups of protein. As a consequence of this complexation, protein and amino acid digestibility were reduced by the inclusion of sorghum and faba bean polyphenols (Jansman *et al*., 1989; Ortiz *et al*., 1993). In the present experiment, apparent ileal digestibility of protein was not affected in chickens at 42 days and slightly at 21 days of age in the highest GSE concentration. These results confirm our previous findings using grape pomace concentrate (Goñi *et al*., 2007; Brenes *et al*., 2008). This lack of effect could be attributed to the low content of polyphenols in the experimental diet to cause detrimental effects.

Regarding relative organ sizes, a higher relative spleen weight was observed in birds fed GSE. That could be explained according to the observations of Magrone *et al*. (2008) who indicated an immunostimulatory activity of red wine polyphenols. In the case of intestinal length, a significant reduction was observed in chicks fed GSE at 21 days of age. Thomas *et al*. (2007) also observed a reduction of intestinal length in birds fed diets containing 0.5% green tea rich in polyphenolic flavonoids, mainly catechins. Likewise, Sehm *et al*. (2006) reported an inhibitory effect on the jejunum villi growth in piglets fed red-wine pomace rich and flavan-3-ol and proanthocyanidins. Nyamambi *et al*. (2007) observed a decrease in small intestinal weight, duodenal villus height and crypt depth in broiler chicks fed sorghum based diets differing in condensed tannin (syn. proanthocyanidins) levels at 21 days of age. We have not an explanation to elucidate the increase observed in intestinal length at 42 days of age.

Given the increasing significance of a potential health beneficial role of GSE proanthocyanidins, there is a need for a fuller understanding of their absorption, metabolism and excretion. The metabolic fate of proanthocyanidins is still elusive as there is conflicting evidence on the absorption and metabolism of the oligomeric and polymeric flavan- 3-ols in humans and animals. Knowledge of the bioavailability and meta-

bolism of polyphenols is necessary to evaluate their biological activity. In the literature reviewed we have not found information relative to polyphenols digestibility in chickens and scarce data are available on polyphenol absorption when these compounds are present in the intestine, together with other dietary constituents. Therefore, in a complex diet, polyphenols are most of the time associated with many constituents that could influence their absorption. In the current experiment, the fecal digestibility of the extractable polyphenols reached values in a range of 57 to 69%. Similar results have been obtained in our lab using dietary grape pomace concentrate in chicken diets (Brenes *et al*., 2008), and in rats by Goñi and Serrano (2005). Wren *et al*. (2002) showed that flavonoid and flavan-3ols metabolites are absorbed in rats through the intestinal lumen and are further metabolised by methylation, oxidation or glucuronic conjugation. There is also evidence in support of absorption of monomeric catechins and proanthocyanidins through the human intestinal caco-2 epithelial cells (Deprez *et al*., 2000; Faria *et al*., 2006). Another study by Tsang *et al*. (2005) reported the absorption and metabolism of catechin and proanthocyanidins up to trimers in urine following the oral intake of GSE.

In the current experiment an increase in antioxidant activity in diet and excreta was observed by the inclusion of GSE at 21 and 42 days of age. This result is similar to those reported in chickens (Goñi *et al*., 2007; Brenes *et al*., 2008) and rats (Goñi and Serrano, 2005). The nutritional effects of polyphenols would be a consequence of the absorbed monomers and aromatic acid, the interaction of unabsorbed polyphenols with components of the intestinal tract, or both. The increase in the antioxidant activity of grape polyphenols in the excreta suggests that part of extractable polyphenols are degraded by intestinal microflora. Goñi *et al.* (2005) reported that intestinal bacteria showed a high capacity to degrade extractable polyphenols in rats. Deprez *et al.* (2000) and Ward *et al.* (2004) also reported that major polyphenolic constituents of grape polyphenols (polymeric proanthocyanidins) were degraded by human colonic microflora into smaller compounds including phenolic acids that could be absorbed and metabolized.

The present study aimed to support the previous results obtained in chicken using grape pomace concentrate and provides evidence that GSE can be incorporated up to 3.6 g kg^{-1} without impair performance, digestive organ size, and protein digestibility. Our results also confirm that polyphenols present in GSE were absorbed at sufficient levels to contribute and modulate the antioxidant activity in diet and excreta. This work has also shown that the phytochemicals present in grapes have antioxidant activity and that this activity in the grape seed extract is related with total phenolic content. On the basis of these observations as well as the previous one (Goni *et al*., 2007; Brenes *et al*., 2008), we concluded that GSE rich in polyphenols could represent an efficient source of antioxidant in the chicken diet. More experiments are in progress to study the effect of dietary GSE in chicken diets on the oxidative stability of meat.

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